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Effects of CO_2 Laser Pulse Duration in Ablation and Residual Thermal Damage: Implications for Skin Resurfacing

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Background and Objectives: **Resurfacing with the CO_2 laser is rapidly gaining acceptance for skin rejuvenation. Advances in CO_2 laser and scanning technology allow for precise tissue removal with minimal thermal damage. High energy CO_2 laser pulses have been widely used effectively to smooth the surface of facial skin; however, pulse duration effects on ablation and thermal damage have not been systematically studied over the millisecond region (0.25–10 ms).**
Study Design/Materials and Methods: **This study characterizes the ablation threshold, heat of ablation, and residual thermal damage in skin resulting from CO_2 laser pulses with a Gaussian beam profile. Mass loss from fresh pig skin was measured with an analytical balance, and residual thermal damage was determined through histology.**
Results: **Pulse durations >1 ms were associated with higher ablation thresholds and localized increased thermal damage.**
Conclusions: **Our results show that although pulse duration is an important determinant in ablation and thermal damage, irradiance is more critical as an independent parameter in predicting the effects of CO_2 laser pulses.**

Key words: irradiance, mass loss, radiant exposure

INTRODUCTION

The creative clinical applications of advances in laser technology have expanded the role of pulsed and rapidly scanned CO_2 lasers in dermatology. Over the past 2 years, CO_2 lasers have been widely promoted for smoothing skin irregularities without scarring. Efficacy has been attributed to high irradiance pulses that ablate with minimal bleeding [1,2]. The devices used clinically are designed to produce minimal thermal damage without charring. By limiting the exposure duration to ~1 ms, heat conduction into the skin is limited during each laser pulse. Although the pulsed CO_2 laser has assumed a major role in skin resurfacing, tissue effects have not been systematically studied over the range of pulse durations used in clinical practice. Therefore, in order to optimize the parameters used in resurfacing, we investigated the in vitro effects of a pulsed CO_2 laser on pig skin using pulsewidths from 0.25–10 ms.

MATERIALS AND METHODS

Laser

A specially modified RF excited pulsed CO_2 laser (Coherent Ultrapulse® Model 5000C, Coherent Laser Group, Palo Alto, CA) produced pulses ranging from 0.25–10 ms. A Gaussian beam profile with a spot size ($\frac{1}{e^2}$) of 1 mm was determined by scanning a 100-μm pinhole through the beam and measuring the transmitted laser energy. En-

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ergies were measured with a modified energy meter (Model J50, Molectron Inc., Portland, OR) attached to an oscilloscope (Model 9400, LeCroy, Spring Valley, NY). Mean radiant exposure was calculated by dividing the delivered pulse energy by the spot size. The 1-mm spot was formed by focusing an 8-mm beam out of the articulated arm through a 100-mm barium fluoride lens. The target plane was reproducibly located by using crossed helium neon laser beams.

Pulsewidths were controlled by a pulse generator (Model PFG 5105, Tektronix, Beaverton, OR) coupled to the RF exciter of the laser. Pulsewidths were verified by a HgCdTe detector and oscilloscope. Radiant exposures for given pulsewidths were controlled by inserting Teflon and plastic film attenuators between the immobilized articulated arm and the focusing lens. Spot diameters were determined both with and without the attenuators to ensure that the attenuators did not alter the 1-mm Gaussian beam profile.

Tissue

Skin was harvested from the trunk of newly euthanized pigs, and an air-powered dermatome (Zimmer, Dover, OH) was used to remove the epidermis and a 150-μm-thick layer of dermis. The skin was stored at 5°C and wrapped in moist saline-soaked gauze. Prior to laser irradiation, the skin was allowed to reach room temperature (25°C). To ensure that tissue degeneration did not affect the laser-tissue interactions, mass loss and thermal damage experiments were conducted within 24 hours of animal euthanasia. The dermal water content was verified by taking three representative dermis samples and allowing for desiccation in a dry oven for 48 hours. The samples were weighed before and after desiccation with an analytical balance (Model AE163, Mettler Instruments, Greifensee, Switzerland).

Mass Loss

Figure 1 shows a schematic diagram of the experimental apparatus. Mass loss was determined using an electronic analytical balance with a precision of 10 μg (Mettler Instruments, vide supra). A personal computer sampled the digital output of the balance 2.4 times per second and stored the data for subsequent analysis. A 2 × 0.5 cm rectangular strip of tissue was mounted on the balance platen. A total of 20 pulses were delivered to each sample at a rate of 0.5 Hz. The sample was moved 2 mm between pulses so that each pulse was delivered to a new spot on the strip. Precise

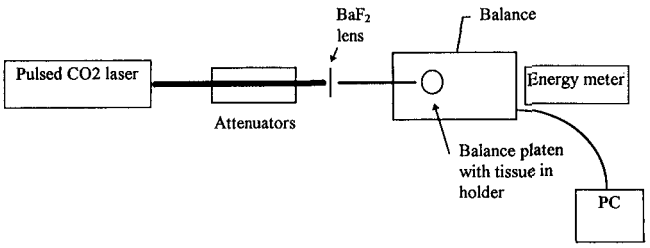


Fig. 1. Schematic diagram of the experimental system.

movement of the sample was possible by placing the balance stop an x-y translator stage. Mass loss was determined over a range of radiant exposures for three pulse durations (0.8, 2, and 10 ms). For each pulse duration, the laser generated a different range of radiant exposures depending on the attenuator configuration. Since pulse durations shorter than 0.8 ms generated only small pulse energies even without attenuation, these pulsewidths were not evaluated for mass loss.

Three samples were irradiated for each pulsewidth-radiant exposure combination. Analysis of covariance was used to show statistical significance between the measured ablation thresholds and heats of ablation for each pulse duration [3].

Residual Thermal Damage

Three skin samples were irradiated for each of the eight pulsewidth-radiant exposure combinations (Table 1). All samples were irradiated with 10 pulses at the same spot at the rate of 1 pulse/s. Multiple pulses were required to observe the cumulative thermal effects of subablative energies at the crater edges for the range of pulse durations. Single pulses were not energetic enough to produce histologically significant damage at the edges of the ablation sites. Prior to histologic processing, the ablation craters were examined for char formation and photographed through an operating microscope (Wild, Heerbrugg, Switzerland). The tissue was fixed in 10% formalin, embedded in paraffin, sectioned, and stained in hematoxylin and eosin. For each pulsewidth-radiant exposure combination, three skin samples were submitted for histology. Up to 100 serial sections were examined for each specimen to ensure that the deepest portions of the craters were observed. Care was taken to orient the specimens in paraffin so that variations due to sectioning angle were minimized. Thermal damage was assessed by light microscopy as the region of collagen tinctorial and polarization change. The depth of damaged tissue was measured with a cal-

TABLE 1. Depth of Residual Thermal Damage at Edge of Crater

	Fluence (J/cm^2)	
Pulsewidth (ms)	7.5	14
0.25	83 μm (8)[a]	94 μm (8.5)[a]
1	89 μm (6)	89 μm (10)
5	110 μm (5)	95 μm (.5)
10	124 μm (15)[b]	116 μm (4.7)[b]

[a]Mean (SD).
[b]$P < .05$ vs. 0.25 and 1 ms.

ibrated eyepiece reticle both at the center and at the edges of the ablation sites.

RESULTS

Tissue

The water contents of the representative dermis samples varied from 61–65%.

Mass Loss

The results from a representative measurement are shown in Figure 2. Because of evaporation, the mass of the tissue decreased spontaneously. The evaporation rate was estimated by computing the linear least-squares fit to the target mass during the 100 seconds prior to laser exposure. The mass loss due to laser ablation was taken to be the vertical distance (i.e., the mass difference) between the best fit line and the mass at the completion of ablation (Fig. 2). The ablation rate was calculated by dividing the mass loss by the number of pulses and expressed in micrograms per pulse.

For the three pulse durations, ablation rates increased linearly with radiant exposure for exposures greater than the ablation threshold. Figure 3 shows the effect of radiant exposure on ablation rate for each pulse duration. The results of a linear least square fit to these data are summarized in Table 2. The ablation rate was considered the dependent variable and the radiant exposure the independent variable. The threshold radiant exposure for ablation was taken to be the radiant exposure for which the linear regression line crossed the x axis. The heats of ablation (determined from the slopes of the regression lines) were not significantly different for the three pulse durations; however, the ablation threshold was significantly greater ($P < 0.05$) for the 10 ms pulse duration vs. the 0.8 and 2 ms pulse durations.

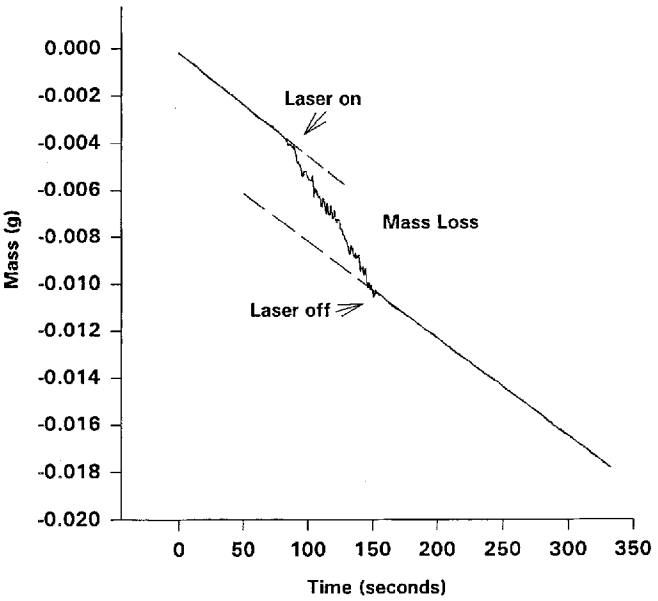


Fig. 2. Representative results from a mass loss measurement. Irradiation with 20 pulses at 90 J/cm^2 removed ~7 mg of tissue. The mass loss before and after the irradiation is due to water evaporation and is compensated for by extrapolating the slope of the curve, after ablation, back to the initiation of irradiation.

Residual Thermal Damage

Review of the gross specimens after irradiation revealed that char was present only for pulse durations >1 ms and then only at the edges of ablation sites (Fig. 4). Three types of changes in collagen staining were noted microscopically. At the most superficial aspects of the pulse sites, a thin zone of basophilic collagen was noted. Deep to this, a less basophilic thicker zone was seen. In some of the biopsies with pulse durations greater than 1 ms, a very fine line of brown char was noted at the periphery of the ablation sites. Irrespective of radiant exposure or pulsewidth, the depths of thermal damage were equal at the center of the craters. However for pulsewidths >1 ms, the edges showed significantly increased microscopic thermal damage (Table 1).

DISCUSSION

This study examines the effects of pulse duration on ablation and residual thermal damage over a range of 0.25–10 ms. Our data show that ablation threshold increased with pulse duration, whereas the differences in heats of ablation for different pulse durations were not statistically significant. We also found that increases in pulse-

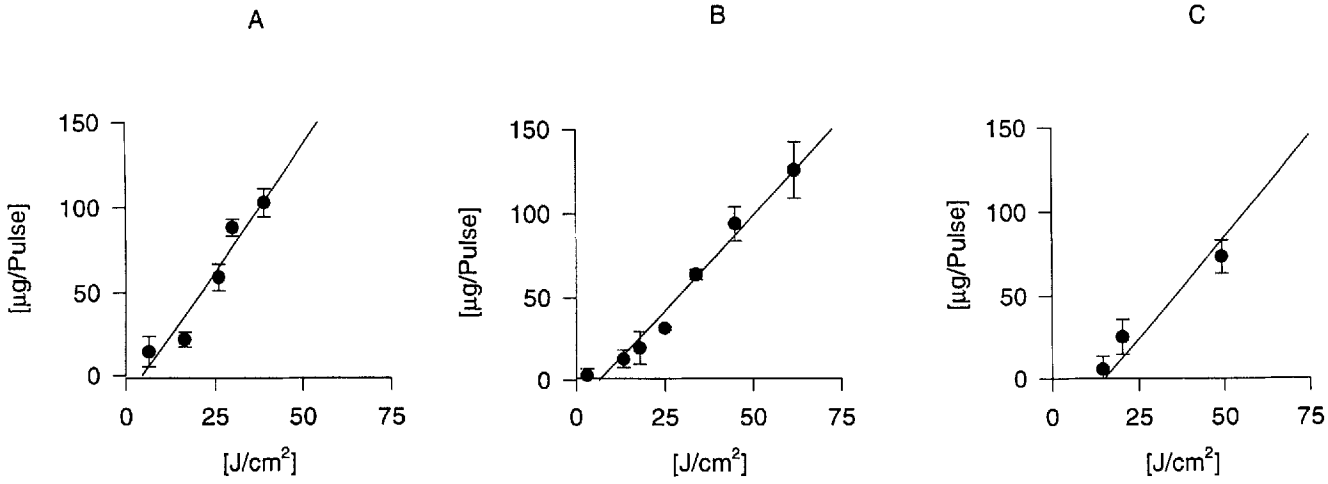


Fig. 3. The mass of pigskin removed per pulse vs. the incident radiant exposure. **A:** 0.8 ms. **B:** 2 ms. **C:** 10 ms. The error bars represent the standard deviation in the measurement of the mass loss at each radiant exposure. Not shown are the 90, 115, and 170 J/cm^2 points for graph 'C'.

TABLE 2. Carbon Dioxide Laser Ablation of Skin

	0.8 ms	2 ms	10 ms	
Linear regression				
Number of samples	15	21	18	
slope	3.0 (2.3–3.6)[a]	2.3 (2.0–2.5)	2.4 (2.3–2.6)	µg · cm^2/J
correlation coefficient (r)	.96	.98	.99	
Derived values[b]				
ablation threshold	5 (0–8.8)[a]	7.4 (1.5–9.2)	15.2 (9.3–21.5)	J/cm^2
heat of ablation[c]	2.9	3.8	3.6	kJ/cm^3

[a]95% confidence intervals are given in parentheses.
[b]See text for details. Irradiated area was 7.85 × 10^{-3} cm^2.
[c]Assumes the density of skin is 1.1 gm/cm^3.

width increased thermal damage at the edges of craters formed by multiple pulses.

One widely used approach to modeling laser ablation uses energy balance [4]. In this model, the ablation rate is expressed as:

$$R = \rho A\,(F - F_{th})/H$$

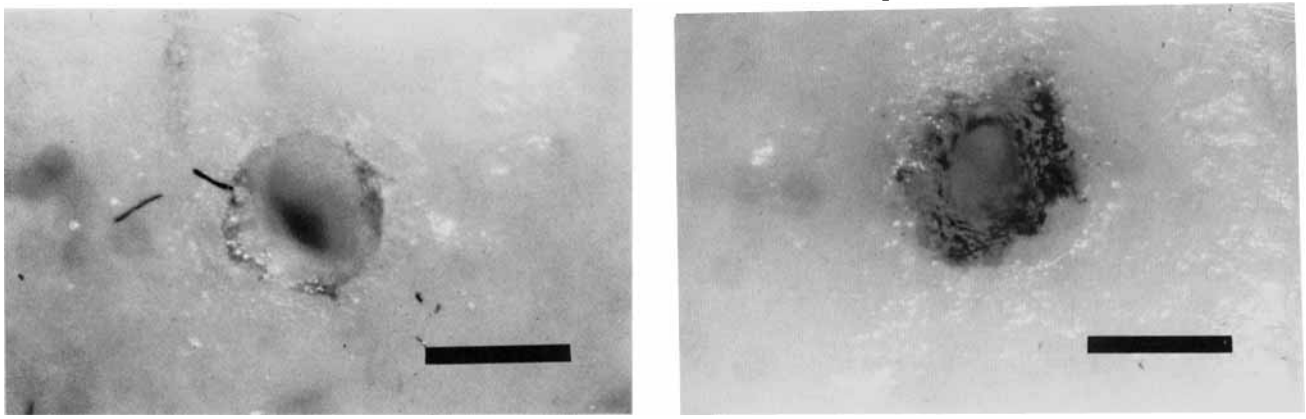
where R is the ablation rate expressed in mass ablated per pulse, ρ is the density of the target, A is the area irradiated by the laser, F is the delivered radiant exposure per pulse, F_{th} is the threshold radiant exposure, and H is the heat of ablation. Using this equation, we calculated H and F_{th} over a range of radiant exposures and pulse durations (Table 2). The increase in ablation threshold at longer pulse durations is caused by thermal diffusion during the pulse. However, heat of ablation did not change as a function of pulse duration since once threshold was reached, the ablation front outpaced thermal diffusion (vide infra).

Our results show that thermal damage at the centers of the craters was independent of pulse duration. This is consistent with previous observations suggesting that during high irradiance ablation (>1,000 W/cm^2), tissue is removed at a rate that effectively "keeps up" with thermal diffusion, regardless of pulsewidth, and therefore residual thermal damage approaches a theoretical minimum of 50 µm [5–9]. Experimental results also showed that thermal damage increased with pulse duration at the crater edges, which is consistent with previous observations that for lower irradiances, thermal damage increases when pulse durations exceed the thermal relaxation time, τ, defined as:

$$\tau = 1/(4\kappa\alpha^2) \qquad \text{(Eq.1)}$$

where α is the absorption coefficient at the laser wavelength, and κ is the thermal diffusivity [6]. For skin, τ has been estimated to be 600–950 µs [6]. In this study, pulse durations spanned the estimated skin thermal relaxation times for 10.6 µm radiation.

The variations in thermal damage across the crater surfaces can be attributed to the Gaussian beam profile of our laser. For example, for the average radiant exposure of 14 J/cm^2 at 10 ms,



a b

Fig. 4. Low magnification (18X) light micrograph of the skin surface following ten-pulse exposure. Average radiant exposure = 14 J/cm^2. **a**: 0.25 ms. **b**: 10 ms. Bar = 1 mm.

irradiance at the center of the crater was 2,800 W/cm^2, whereas 400 μm from the center (where an increase in thermal damage was observed histologically), the irradiance decreased to 750 W/cm^2. In contrast, for the same average 14 J/cm^2 radiant exposure delivered in only 0.25 ms, the center irradiance was 112,200 W/cm^2 and the edge irradiance 31,000 W/cm^2. The observations of increased thermal damage at the crater edges can be predicted by computing the ablation and heat conduction velocities for each case. The velocity of ablation (v_a) is simply:

$$v_a = \frac{I}{H_a} \qquad \text{(Eq.2)}$$

where I is the local irradiance and H_a is the heat of ablation using the average of the calculated heats of ablation from our mass loss experiments for H. Using [Eq 2], the ablation velocities at the edges of the craters for the 10 and 0.25 ms pulsewidths are 0.2 and 9.1 cm/s, respectively.

To achieve minimal thermal damage, v_a must exceed an effective heat conduction "velocity", v_c, given by:

$$v_c \approx \frac{d}{\tau_d} \qquad \text{(Eq.3)}$$

where d is the minimum depth of histologically identifiable thermal damage (~80 μm in our experiment) and τ_d is time required for heat conduction over this distance. Because $\tau_d \approx d^2/4\kappa$, the term for v_c reduces to: $v_a \approx 4\kappa/d$, which has a value of ~0.65 cm/s. Therefore, for any CO_2 laser that achieves $v_a \geq 0.65$ cm/s, minimal thermal damage will be observed.

At the edges of the 10 ms craters, the ablation velocity was outpaced by thermal diffusion, whereas for the 0.25 ms craters, the ablation front was moving well ahead of thermal diffusion. This explains the increased thermal damage at the crater edges for the 10 ms pulses. The increases are small on a percentage basis, e.g., 116 μm vs. 94 μm for the 10 and 0.25 ms crater edges, respectively (Table 1, 14 J/cm^2). However, they are statistically significant and, more importantly, are the quantitative differences predicted by the solution of the general heat equation for collagen denaturation for the respective power densities [7].

We can calculate the threshold irradiance for achieving minimal thermal damage by setting:

$$v_a = v_c$$

Substituting Eqs. 2 and 3, and letting:

$$I = I_{th}$$

we have:

$$\frac{I_{th}}{H_a} = \frac{d}{\tau_d}, \text{ which gives}$$

$$I_{th} = \left(\frac{d}{\tau_d}\right) H_a \qquad \text{(Eq.4)}$$

Inserting the experimentally determined values for H_a, d, and τ_d

$$I_{th} = 2200 \text{ W/cm}^2$$

This value, as a threshold irradiance for predicting increased thermal damage, is consistent with our observations, since as a rule the center irradiances were near or above 2,200 W/cm^2 (even for the 10 ms pulse in the example above, the center irradiance was 2,800 W/cm^2), whereas at the edges, irradiances decreased well below this critical number for the 5 and 10 ms pulse durations.

Thus the mass loss and thermal damage observed in our study are consistent with energy balance and heat transfer theory.

Clinical Implications

The study findings relate clinically to the role of ablation in resurfacing. Conventional teaching is that wrinkle shoulders are ablated and troughs are left untreated so that the skin is eventually smoothed [2]. However, our study suggests that other mechanisms are involved. For example, in practice the pulsed CO_2 laser is regularly used with a 3 mm spot size (collimated beam), pulse duration from 0.5–1 ms, and fluences from 3.5–7.1 J/cm^2. Therefore, even with the maximum pulse energy, the clinician operates just above ablation threshold, where according to our observations, ablation depth per pulse (for 7.5 J/cm^2) was only 35 μm. Since an average visible wrinkle from the periorbital area measures 300 μm (measurements in our lab with skin replicas), 7–8 passes of the laser would be required to eliminate the defect if ablation were the only mechanism for clinical improvement. But in clinical practice, usually only 2–3 passes are used, yet clinical improvement is observed. Also, even with subablative fluences, clinical improvement is the rule [1,2].

Besides ablation, a potential mechanism in resurfacing is the immediate contraction of irradiated areas observed both in vitro and in vivo with the pulsed CO_2 laser. This contraction of tissue most likely represents desiccation and collagen shrinkage, both recognized results of tissue heating [10]. In our clinic patients, we have observed that wrinkles that persist just after irradiation will often resolve over 6 months after treatment; thus biological remodeling may represent a third complementary mechanism to ablation in clinical improvement [11].

In this study we observed thermal damage differences as a function of pulse duration that resulted from ten overlapping pulses. In practice it is unusual to make more than four or five laser passes, and it would be highly unlikely that all of these would be overlapping. Thus there may be an extended range of pulse durations for which thermal damage will be limited and tolerable. It follows that, based on thermal damage by histology, a 10 ms laser pulse might result in wound healing similar to a 1 ms laser pulse. However, it cannot be assumed that the depth of injury is identified solely through routine histology [12, 13]. Longer pulse durations may injure deeper tissue through exposure to temperatures not capable of causing bulk denaturation of the extracellular matrix. Where there is ablation, the pulse duration is not critical, since there is a rapid temperature drop just below the moving ablation front [5,7]. Indeed, this may be precisely why ablation is desirable in resurfacing; with ablation, energy can be deposited in a small zone of tissue so that "just" the right amount of collagen is denatured for hemostasis and optimal wound remodeling. Without ablation, the temperature profile under the skin decays more slowly, so that to achieve enough thermal damage for hemostasis and collagen remodeling, higher temperatures may extend unacceptably deep into the dermis, and scarring may result. Tissue viability stains have confirmed that histologically "invisible" tissue injury does occur in low irradiance CO_2 laser applications [12]. Thus it may be clinically advantageous to restrict pulse durations to less than the thermal relaxation time (and therefore maintain a critical irradiance) so that thermal damage is consistent for the both ablation center and crater edges.

In conclusion, to achieve ablation with minimal thermal damage, pulse duration should be $\leq$1 ms for radiant exposures between 7.5 and 14 J/cm^2. Longer pulses were shown to focally increase thermal damage and increase ablation threshold. Since the effects of pulse duration on biological responses were not examined, future studies should investigate depth of necrosis, wound healing, and scarring as a function of pulsewidth. Also, the role of ablation in skin resurfacing should be reappraised, and complementary mechanisms should be studied.

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